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Dyeing properties, synthesis, isolation and characterization of an in situ generated phenolic pigment, covalently bound to cotton

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ABSTRACT

Oxidation of colourless dye precursors with laccase enzyme provided simultaneous "in situ" generation and fixation of a pigment on amino groups pre-functionalized cotton fabric. Aromatic amine moieties of 2,5-diaminobenzenesulfonic acid introduced onto tosylated cotton were coupled and copolymerised with a phenolic compound catechol into coloured product covalently fixed on the fabric upon oxidation with laccase. The controlled amination of cellulose in a first step and subsequent colouration allowed for up to 95% pigment fixation on the fabric. Electrochemical studies were performed to elucidate the mechanism of the pigment formation. The pigment was further isolated from the acid hydrolysate of the dyed cellulose fabric to confirm the covalent fixation and to further elucidate the pigment structure by means of FTIR, MS, ^{1}H and ^{13}C NMR analysis. An oligomeric pigment has been identified composed by up to six phenolic units.

1. Introduction

The use of enzymes to synthesize colourants "in situ" could be an efficient way for textiles dyeing at mild, in terms of chemicals, pH, temperature and process conditions. Laccases are oxidoreductase enzymes that oxidize colourless aromatic compounds such as phenols, aminophenols and diamines [1,2] to aryloxy radicals capable to undergo further non-enzymatic oxidation resulting in coloured dimeric, oligomeric or polymeric compounds. This enzymatic approach has been already reported for dyeing of protein fibres [3–7] and in general claimed in the patent literature for dyeing of any textile substrate, however, without providing clear examples and mechanism for this [8]. In previous works [9,10] we demonstrated for the first time a permanent colouration of cotton fabrics based on the oxidative coupling of catechol and 2,5-diaminobenzenesulfonic acid (2,5-DABSA) using laccase enzyme. The lack of chemical bond formation between the laccase-generated pigments and cellulose was compensated by optimisation of the concentration of the pigment precursors, yielding up to 70% pigment fixation due to the formation of an insoluble coloured product.

Another strategy to improve pigment fixation is a pre-functionalisation, e.g. amination of cellulose reported recently as an approach for subsequent enzyme-catalysed grafting of polycatechol [11]. Dyeing with C.I. Reactive Black 5 followed by reduction of the azo-bonds in dye structure with sodium hydrosulfite was claimed to ensure the amine functionalisation of cotton. However, quantitative evidence for the degree of substitution in cellulose and therefore reproducibility of the functionalisation and dyeing was not provided.

The present work aims on improving the colour strength and fixation in laccase-assisted colouration of cotton fabrics by means of controlled pre-functionalisation of cellulose with 2,5-DABSA. The aromatic amine was introduced to a previously tosylated fabric through nucleophilic displacement of the tosyl groups. This aminated cellulose allows for the covalent fixation of the "in situ" generated from catechol and amine pigment, upon oxidation with laccase. Electrochemical studies [12,13] in addition to HPLC, MS–MS, ^{1}H and ^{13}C NMR analysis were performed to elucidate the mechanism of the dyeing process and the structure of the pigment grafted on the fabric.

2. Experimental

2.1. Materials

Samples of bleached, knitted, 100% cotton fabric were supplied by Tinfer S.L. Spain. *p*-Toluenesulphonyl chloride, pyridine (absolute, over molecular sieve (H_2O ≤0.005%)), 2,5-diaminobenzenesulfonic acid, acetone, hydrochloric acid and catechol were purchased from Sigma–Aldrich. Laccase (EC 1.10.3.2 *Trametes* sp. laccase, Laccase L603P; 0.125 g prot. per gram solid) was provided by Biocatalysts, UK.

2.2. Cotton modification

Cotton fabric was tosylated [14,15] with *p*-toluenesulphonyl chloride (tosyl chloride) according to Scheme 1a. Briefly cotton fabric (3 g) were incubated in pyridine (35 mL) for 3 h in a thermostated laboratory shaker at 100 rpm and at

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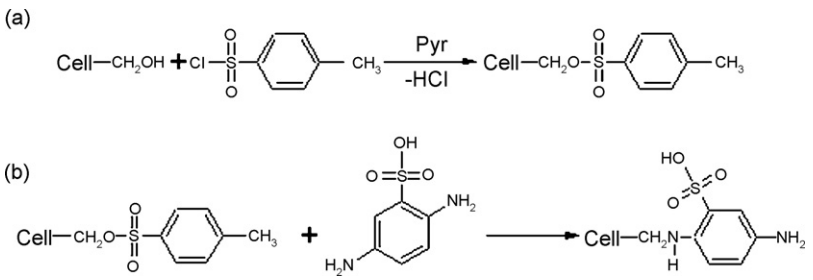

Scheme 1. Schematic representation of (a) cotton tosylation and (b) nucleophilic attack of 2,5-DABSA on tosylated cotton.

room temperature. Thereafter, a solution of *p*-toluenesulphonyl chloride (24 g) in pyridine (40 mL) was added and the reaction was carried out at room temperature for another 3 h at 100 rpm. The fabric was washed first with acetone and then extensively with 0.005 M HCl solution. Tosylated cotton was stored at 4 °C in a solution of 0.005 M HCl until it was used.

Distilled water washed tosylated fabric (0.25 g) was placed in 0.015 M 2,5-diaminobenzenesulphonic acid solution during 3 h at 75 °C and 100 rpm. Samples were washed at boil with non-ionic surfactant Cotemol NI (Color Center, Spain) to remove any unreacted amine and *p*-toluenesulfonic acid. The amount of tosyl groups on fabrics was determined spectrophotometrically (Thermo Helios γ spectrophotometer), by measuring the *p*-toluenesulfonic acid absorption at 261 nm after hydrolysis of the tosyl moieties in 3 M solution of NaOH for 24 h [15].

2.3. Enzymatic colouration

The 2,5-DABSA modified cotton samples were incubated with 0.004–1 M catechol in 0.1 M sodium acetate/acetic acid buffer pH 5 in an Ahiba Spectradye-Datacolor apparatus at 40 rpm and 40 °C for different times. The dyeing was started with the addition of 0.1 g L^{-1} laccase. After treatment, samples were washed at boil with non-ionic surfactant Cotemol NI to remove the unfixed pigment. The colour strength of the samples (*K*/*S*) was evaluated at 400 nm using a reflectance measuring apparatus Spectraflash 300 Datacolor (LAV/Spec. Incl., d/8, D_{65}/10). *K*/*S* is the Kubelka-Munk relationship, where *K* is an adsorption coefficient and *S* is a scattering coefficient. The pigment fixation (*F*) was calculated as $\%F = ((K/S)_a/(K/S)_b) \times 100$ from the *K*/*S* data after (a) and before (b) washing of the dyed samples. The *K*/*S* determination was carried out in triplicate on three cotton samples.

2.4. Dyed cotton hydrolysis

Dyed cotton sample (0.5 g) was hydrolysed in 72% H_2SO_4 (5 mL) for 1 h at 30 °C prior to the HPLC, MS-MS and NMR analysis. Thereafter, the total volume was brought to 148.67 g with distilled water and the mixture was incubated for 3 h at 90 °C and 100 rpm constant agitation.

2.5. DRIFT measurements

Diffuse reflectance infrared Fourier transform (DRIFT) spectra of the modified and dyed samples over the 500–4000 cm^{-1} range were collected by a PerkinElmer Paragon 500 FT-IR spectrometer, performing 100 scans for each spectrum.

2.6. Elemental analysis

Elemental analysis of the fabric after 2,5-DABSA modification was carried out by combustion of the sample at 1200 °C in oxygen atmosphere and posterior quantification of C, H, N and S in a gas chromatograph. Analysis data are mean value of three samples.

2.7. Fabric hydrophilicity

Hydrophilicity of fabric was determined by measuring the absorption time of a water drop on the surface of the fabric, as previously reported in literature [16].

2.8. HPLC analysis

HPLC analysis was carried out using an Agilent 1200 series HPLC system equipped with an Aminex HPX87H column and an Agilent 1200 series Refraction Index detector. The mobile phase was 5 mM H_2SO_4 with a flow rate of 1 mL min^{-1}.

2.9. NMR analysis

^1H and ^{13}C NMR analyses were run in a Varian Mercury 400 apparatus using DMSO-d_6 as a solvent at 293 K.

2.10. MS analysis

MS analysis was performed in an Applied Biosystems API 365 mass spectrometer using ESI ionization in the positive mode and a scan range from m/z = 100 to 2000.

2.11. Electrochemical experiments

Voltammetric measurements were performed using a μAutolab Type III (Eco-Chemie) potentiostat/galvanostat controlled by Autolab GPES software version 4.9. All the experiments were carried out in a 20 mL Metrohm cell with a three-electrode configuration. The working electrode was a glassy carbon with a surface diameter of 3 mm (Metrohm). The counter and reference electrodes were platinum (Metrohm) and Ag/AgCl (Metrohm) electrode, respectively. The renewal of the glassy carbon surface was achieved by polishing with 1.0 and 0.3 μm alpha-alumina (Micropolish, Buehler) on a microcloth polishing pad (Buehler), followed by washing in an ultrasonic Selecta bath for 2 min. For the experiments with cotton samples, the working electrode was put in contact with the textile inside the electrochemical cell.

3. Results and discussion

3.1. Surface modification of cotton fabrics

The main difficulty in the dyeing of cotton "in situ" with laccase lies in the lack of affinity of oxidative enzyme-generated dyes towards cellulose. Normally, the conventional dyes for cotton bear reactive groups and are covalently grafted on the fibres at alkaline conditions. In our recent works [9,10] a laccase mediated process using catechol and 2,5-DABSA was successfully applied for dyeing of cotton. Up to 70% pigment fixation was achieved working with 10-fold excess of catechol (the compound that upon oxidation with laccase forms less soluble products). Further step in the engineering of a laccase-assisted process for dyeing of cellulose substrates would be to graft one of the pigment precursors covalently on the fabric, and thereby to ensure the permanent fixation of the enzymatically generated pigment. To achieve this, the cotton fabric was first tosylated and then the leaving ability of tosyl group was exploited to convert the C6 from cellulose into a good electrophile prone to participate in SN_2 reactions with 2,5-DABSA, according to Scheme 1b. The degree of tosylation of the fabric, determined spectrophotometrically, showed 60 μmol of tosyl per gram of fabric, which means a degree of cellulose substitution (DS) of 0.011.

The DRIFT spectra (Fig. 1) for unmodified cotton (dotted line) and for tosylated cotton (black) showed a clear difference in the 850–650 cm^{-1} area. In the spectrum of tosylated cotton four new peaks (at 832, 815, 751 and 693 cm^{-1}) appeared. The peak at 832 cm^{-1} could be assigned to S–O bond, while the other three peaks come from the substituted aromatic ring. Aromatic C–C bonds appear at 1658 and 1598 cm^{-1}. In the insert figure, correspondence in some peaks between *p*-toluenesulfonic acid and tosylated cotton spectra can be observed.

Little differences were expected between the spectra of 2,5-DABSA-treated cotton and the tosylated one, since in both samples S–O bonds and aromatic rings are present. Furthermore, the peaks for the amino groups from 2,5-DABSA appear overlapped by hydroxyl groups from cellulose. The decrease of the peaks at 1658